



TITLE:

Kinetic model of thermophilic L-lactate fermentation by *Bacillus coagulans* combined with real-time PCR quantification.

AUTHOR(S):

Hidaka, T; Horie, T; Akao, S; Tsuno, H

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Table 1 Reaction rate and mass balance for model development

(Reaction rate)
Degradation of particulate carbohydrate (gCOD/(L·hr)):
$R_1 = k_1 \cdot \frac{C_c/X}{K_{C_c} + C_c/X} \cdot X$
Degradation of soluble carbohydrate (gCOD/(L·hr)): $R_2 = k_2 \cdot \frac{S_c}{K_{S_c} + S_c} \cdot X$
Lactate fermentation (gCOD/(L·hr)):
$R_3 = k_3 \cdot \frac{S_G}{K_{S_G} + S_G \cdot \left(1 + \frac{S_G}{K_{ES}}\right)} \cdot \frac{K_{EI}}{K_{EI} + S_L} \cdot \frac{K_{EIN}}{K_{EIN} + S_N} \cdot \left(1 - \frac{\max(0, S_L - S_{Lct})}{S_{Lmax} - S_{Lct}}\right) \cdot X$
Self-degradation (gCOD/(L·hr)): $R_4 = k_4 \cdot X$
(Mass balance)
$\frac{dC_c}{dt} = -R_1$
$\frac{dS_c}{dt} = R_1 - R_2$
$\frac{dS_G}{dt} = R_2 - R_3$
$\frac{dS_L}{dt} = (1 - Y) \cdot R_3$
$\frac{dX}{dt} = Y \cdot R_3 - R_4$
k_1 , Rate constant for R_1 (gCOD/(gCOD·hr)); k_2 , Rate constant for R_2 (gCOD/(gCOD·hr)); k_3 , Rate constant for R_3 (gCOD/(gCOD·hr)); k_4 , Rate constant for R_4 (1/hr); K_{C_c} , Half saturation constant for R_1 (gCOD/L); K_{S_c} , Half saturation constant for R_2 (gCOD/L); K_{S_G} , Half saturation constant for R_3 (gCOD/L);

K_{ES} , Inhibition parameter of glucose for R_3 (gCOD/L); K_{EI} , Inhibition parameter of lactate for R_3 (gCOD/L); K_{EIN} , Inhibition parameter of NaCl for R_3 (gNaCl/L); S_{Lct} , Lactate concentration when inhibition of lactate starts (gCOD/L); S_{Lmax} , Maximum lactate concentration (gCOD/L); Y , Yield constant for R_3 (gCOD/gCOD);

Table 2 Experimental condition

Run		1a	1b	2	3	4	5	6	7	8
Glucose	(g/L)	10	10	10	10	50	50	100	0	0
Lactate	(g/L)	-	-	20	-	-	6	-	-	-
NaCl	(g/L)	-	-	-	20	-	-	-	-	-
Kitchen garbage	(L/L)	-	-	-	-	-	-	-	0.5	0.25
Banana peel	(L/L)	-	-	-	-	-	-	-	-	0.17

Table 3 Characteristics of kitchen garbage

	Kitchen garbage		Banana peels	
	Total	Soluble	Total	Soluble
TS (g/L)	190	—	121	—
SS (g/L)	86	—	65	—
VTS (%)	96	—	88	—
COD (g/L)	201	114	79	42
Carbohydrate (g/L as glucose)	115	96	44	33
Protein (g/L as albumin)	20.3	3.7	4.5	1.6
T-N (mgN/L)	3.4	1.2	1.4	1.0
pH (-)	5.1	—	5.5	—

Table 4 Summary of each batch experiment

Run		1a	1b	2	3	4	5	6	7	8
Time when fermentation started	(h)	6	6	20	11	6	7	9	12	-
Lactate concentration at the end	(g/L)	8.6	9.3	29	9.3	45	48	52	35	21.5±0.4
Lactate production ratio from carbohydrate	(-)	0.86	0.93	0.74	0.89	0.90	0.84	0.52	0.54	0.59
OP	(%)	100	100	100	100	100	100	98.3	98.5	99.3

Table 5 Model parameters

parameter	value
k_1	0.1 (gCOD/(gCOD·hr))
k_2	80 (gCOD/(gCOD·hr))
k_3	12.8 (gCOD/(gCOD·hr))
k_4	0.01 (1/hr)
K_{C_c}	1.0 (gCOD/L)
K_{S_c}	1.0 (gCOD/L)
K_{S_G}	3.1 (gCOD/L)
K_{ES}	180 (gCOD/L)
K_{EI}	5.11 (gCOD/L)
K_{EIN}	30 (gNaCl/L)
S_{Lct}	40 (gCOD/L)
S_{Lmax}	56 (gCOD/L)
Y	0.12 (gCOD/gCOD)

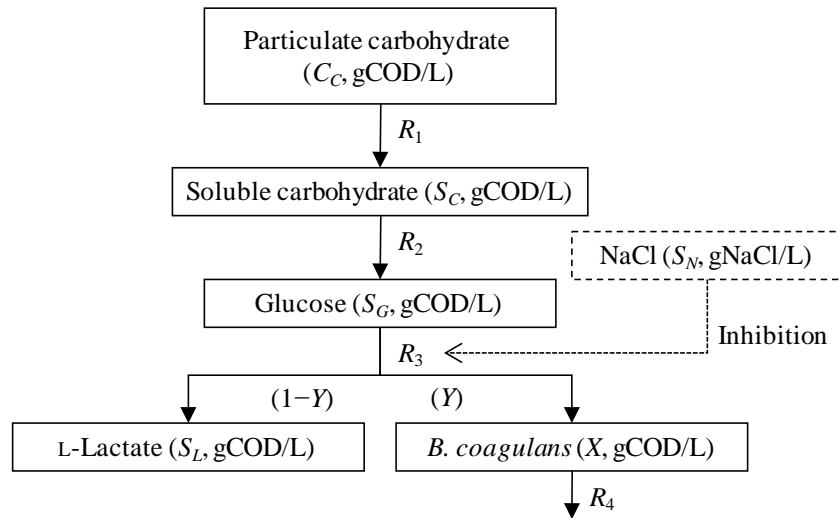


Figure 1 State variables and transformation paths

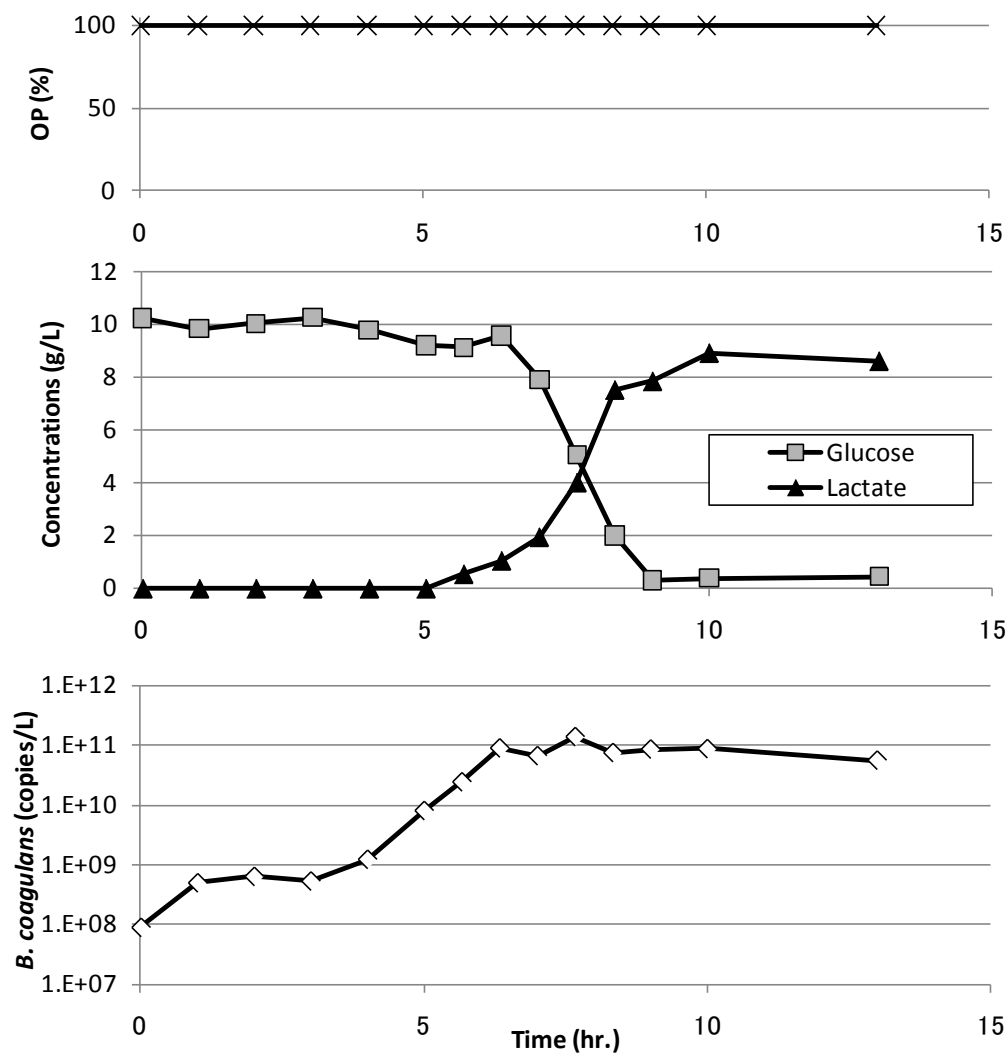


Figure 2 Results of Run 1a

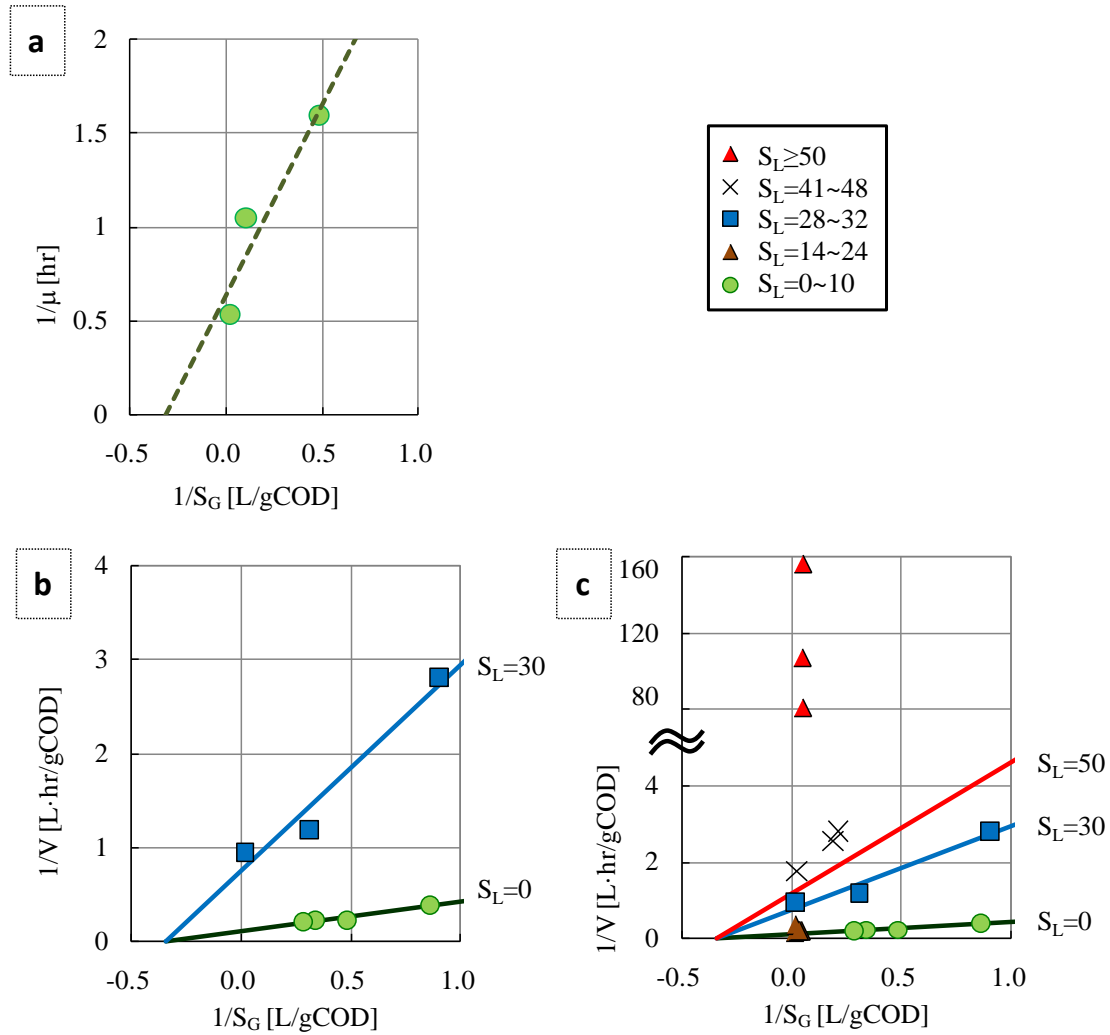


Figure 3 Lineweaver-Burk plot for accumulated lactate inhibition depending on glucose concentrations. μ is specific growth rate (1/hr), V is measured glucose consumption rate (gCOD/(L·hr)), S_G and S_L are concentrations of glucose and lactate, respectively. Solid lines are calculated by the non-competitive inhibition model equation when S_G is 0, 30, or 50 (gCOD/L).

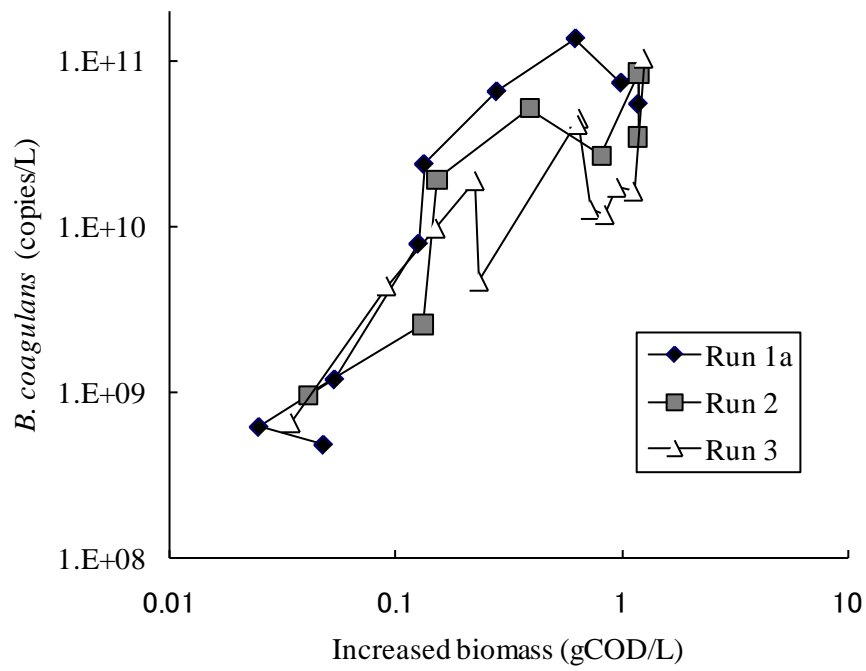


Figure 4 Relationship between increased biomass and real-time PCR analysis

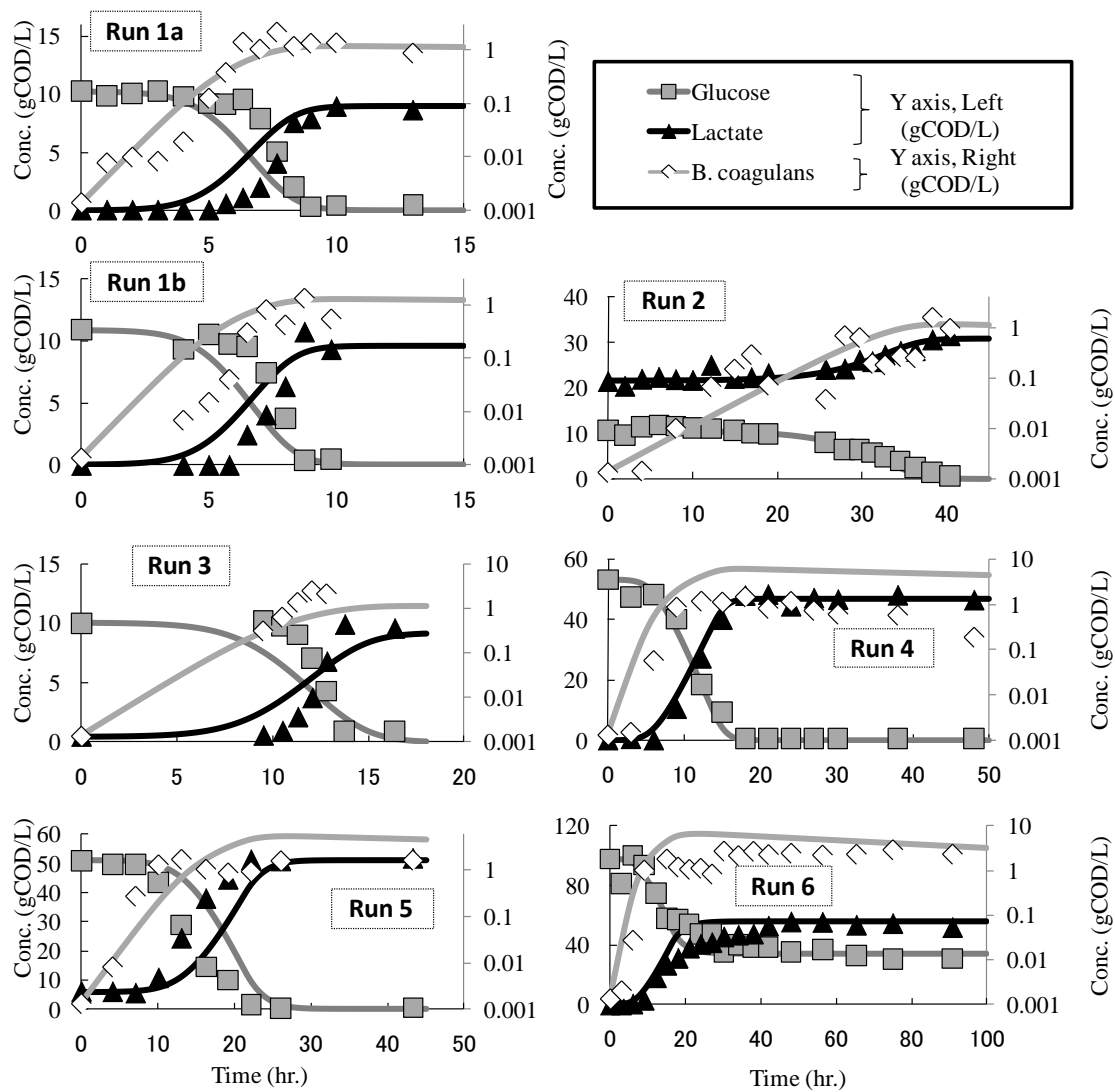


Figure 5 Time courses of the experimental and calculated results in Run 1~Run 6. Plots are experimental data and lines are simulated results.

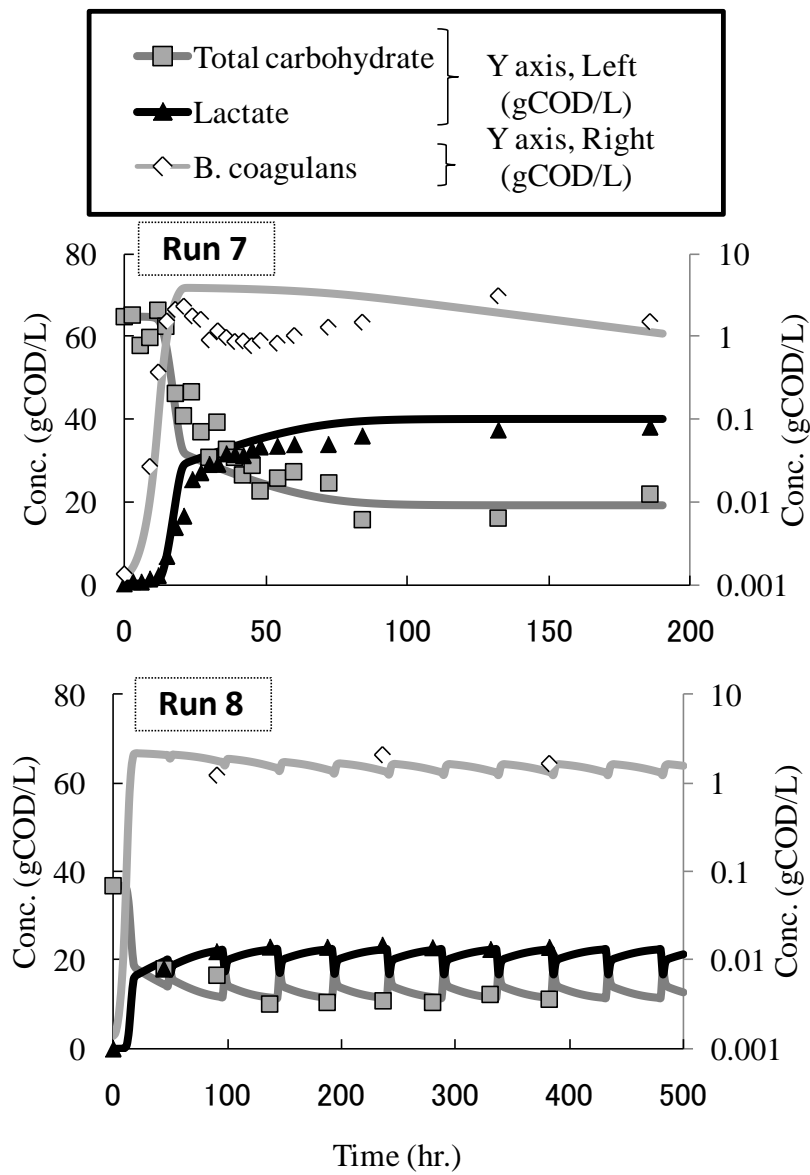


Figure 6 Time course of the experimental and calculated result in Run 7 and Run 8. Plots are experimental data and lines are simulated results.